


REMARKS

Applicants submit this Amendment to correct the required references to SEQ ID NOS of the Sequence Listing filed concurrently herewith, and to indicate the insertion point for the Sequence Listing.

Applicants respectfully request examination on the merits of this application.

Respectfully submitted,

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Date


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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ATTY. DOCKET NO: 023032/0108

In re Patent Application of
ROBERTS, Joseph et al.

Serial No.: 09/842,628

Group Art Unit: 1648

Filing Date: April 27, 2001

Examiner:

For: **GENETICALLY ENGINEERED GLUTAMINASE AND ITS USE IN
ANTIVIRAL AND ANTICANCER THERAPY**

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Marked up version showing changes made to the paragraph bridging pages 7 and 8:

Figure 1 shows the nucleotide and deduced amino acid sequence of the *Pseudomonas 7A* glutaminase gene. The top strand of the coding DNA sequence (SEQ ID NO: 1) is shown from 5' - 3'. The numbers shown indicate nucleotide base pairs. The deduced peptide sequence is shown below the DNA sequence (SEQ ID NO: 2). The engineered N-terminal methionine residue is not shown.

Marked up version showing changes made to page 8, paragraph 2:

Figure 2 depicts the sequencing strategy for the *Pseudomonas 7A* glutaminase gene.

Figure 2A: Map of the *P7A* glutaminase showing selected restriction sites, the shaded area depicts the region encoding the actual gene product. Hatch marks represent 100 bp. Arrows below this figure show the approximate positions and orientations of sequencing primers with their accompanying names. The arrows with stops indicate the extent and direction of individual sequencing experiments. Figure 2B: Names, sequences, and coordinates of

sequencing primers (SEQ ID NOS: 3-11, respectively in order of appearance) are shown.

Numbering is from the AAG encoding the N-terminal lysine residue.

Marked up version showing changes made on page 11, paragraph 2:

The *P7A* glutaminase sequence (SEQ ID NO: 1) which is disclosed here can be used to identify similar sequences encoding similar proteins. (See Watson, J.D. *et al.*, in "Molecular Biology of the Gene." Benjamin/Cummings Publishing Company Inc., Menlo Park CA, Vol. I, p. 608 (1987)). For example, Southern hybridization experiments can be carried out in which prokaryotic or eukaryotic organismal DNA is probed with all or part of the glutaminase gene of the present invention. Typically probes contain at least about 15 bases of the glutaminase sequence in order to ensure that other non-related sequences do not hybridize. Sufficiently high temperature and low salt concentrations further reduce hybridization to non-related sequences. Using such techniques, homologous genes have been found to *dnaA* of *E. coli* in *Pseudomonas putida* (Ingmer and Atlung, Mol. Gen. Genet. 232, 431 (1992)) and to *ras* in a variety of eukaryotic organisms (Matsui, Gene 76:313 (1989) and Hori, Gene 105:91 (1991)). There is a high probability that DNA sequences that hybridize to the *P7A* glutaminase DNA represent genes encoding enzymes of similar function. Genes which are isolated by this technique can be expressed, and the enzymes can be tested to determine if they share the desirable characteristics identified for the *P7A* glutaminase.

Marked up version showing changes made on page 21, paragraph 1:

This example demonstrates the identification of a clone containing the sequence coding for *Pseudomonas 7A* glutaminase and determination of its nucleotide sequence (SEQ ID NO: 1).

Marked up version showing changes made on page 23, paragraph 1:

Table 1. OLIGONUCLEOTIDE PROBES USED FOR DETECTING THE GLUTAMINASE GENE

Peptide Sequence (1-5)	(portion of SEQ ID NO: 2) NH ₂ -Lys-Glu-Val-Glu-Asn
Probe A (14-mer x 32)	(SEQ ID NO: 12) AA (AG) GA (AG) GT (TCAG) GA (AG) AA
Peptide Sequence (161-166)	(portion of SEQ ID NO: 2) Met-Asn-Asp-Glu-Ile-Glu
Probe B (18-mer x 48)	(SEQ ID NO: 13) ATGGA (TC) GA (TC) GA (AG) AT (TCA) GA (AG)
Peptide Sequence (332-36)	(portion of SEQ ID NO: 2) Ile-Phe-Trp-Glu-Tyr-COOH
Probe C (14-mer x 12)	(SEQ ID NO: 14) AT (TCA) TT (TC) TGGGA (AG) TA

In order to confirm the identity of the putative PGA clone, the region of homology to the probes used for screening was localized by Southern blot analysis, and the appropriate fragments were partially sequenced. This analysis identified a 1.1 Kb *Sal*I fragment which hybridized to probe A, and a 1.5 Kb *Sal*I fragment which hybridized to probe B. This indicated that there was a *Sal*I site within the gene, and that sequencing from this site would immediately confirm the identity of the gene as PGA by comparing the nucleotide sequence with the known amino acid sequence. Sequencing of the 1.1 kb *Sal*I fragment showed that this fragment encodes the N-terminal 42 amino acids of the glutaminase.

Marked up version showing changes made on page 26:

Table 3: Oligonucleotides Used in Construction
of a High Level Expression Plasmid

Primers for thermocycle amplification mutagenesis of the glutaminase:

N-terminus

(SEQ ID NO: 15) GCCGGATACCA TATGAAGGAA GTGGAGAACC AGCAG

Internal SalI site

(SEQ ID NO: 16) GCGCGGATCC GTCGACGCCA ACCTTGGCAG

Mutagenized N-terminus of the glutaminase

(SEQ ID NO: 17) GGATCCAT ATG AAG GAA GTG GAG AAC

(SEQ ID NO: 18) Met Lys Glu Val Glu Asn. . .

Oligonucleotides for *tac* promoter

top:

(SEQ ID NO: 19) AGCTTACTCC CCATCCCCCT GTTGACAATT AATCATCGGC TC
GTATAATGTG TGGAATTGTG AGCGGATAAC ATTTACACAG AGGAAACAG

bottom:

(SEQ ID NO: 20) GATCCTGTTT CCTGTGTGAA ATTGTTATCC GCTCACAATT CCACACA
TTATACGAGC CGATGATTAA TTGTCAACAG GGGGATGGG AGTA

Filled in product of pME18

(SEQ ID NO: 21) *lacO*

5' AATTGTGAGCGGATAACAATTTCACAC

AGGA AAC

S.D.

S.D.

AGGATCCATAT ATG AAG

(SEQ ID NO: 22) Met Lys

GAA GTA GAG AAC 3'

GLU Val Glu Asn